

NASA Grant NAG10—0142 “Starch metabolism in space-grown soybean seedlings”

1994-1999 Final Report

Principal Investigator: James A. Guikema, Kansas State University
Co-Investigators: Jan E. Leach, Kansas State University
Christopher Brown, Kennedy Space Center

History and scope of the award

This NASA grant was originally written with **Dr. James A. Guikema** as the principal investigator and **Dr. Christopher Brown** as the co-principal investigator. It was written and approved to support the summer research activities of Emmanuel Hilaire in conjunction with the Plant Space Biology Program at the Kennedy Space Center. This graduate student was co-advised by Drs. James A. Guikema (Kansas State University) and Christopher Brown (Dynamac/Kennedy Space Center). Academic year activities were performed in the KSU Guikema laboratory, while summer research activities were performed in the KSC Brown laboratory. The focus of this research was the study of sugar metabolism in soybean plants that had been in a clinorotation condition. As a result of this research, Mr. Hilaire was awarded a Ph.D. from Kansas State University, and he continued with the SOYPAT project outlined below.

The grant was amended following a reiteration of the peer review process to include activities involved with the Cooperative Ukrainian Experiment, which flew aboard STS-87 during the last part of 1997. The listing of co-principal investigators was amended to include **Professor Jan E. Leach** of the Kansas State University Department of Plant Pathology. The budget was amended to support (1) the preflight activities, (2) the proof-of-concept activities performed at the Kennedy Space Center (both the PVT and the SVT), (3) activities to support the flight experiment aboard STS-87 (November – December, 1997), and (4) a detailed assessment of the results obtained from the mission (from December, 1997, until the end of the award period).

It is apparent, therefore, that the scope of activities was broadened greatly after the onset of the award. This broadening added two major research foci:

- B-PAC: Photosynthetic activity of *Brassica rapa* following germination and growth in the microgravity environment of orbital spaceflight. Ukrainian collaborators in this experiment were Drs. Elizabeth Kordyum, Svetlana Kochobey, and Nadja Adamchuck. Major finding showed that PSI is severely impacted following spaceflight.
- SOYPAT: The effects of microgravity on plant pathology, focusing on the interaction of a fungal root pathogen with soybean. Ukrainian collaborators on this experiment were Drs. Elizabeth Kordyum and Olena Nedukha. Major finding showed that spaceflight-grown plants were clearly more susceptible to invasion by pathogen.

Substantial investment and activity was also focused on the training of the astronaut team to conduct these experiments during orbital spaceflight.

The interaction of gravity and sugar metabolism in soybean

This research focused on the understanding of cellular physiology of plants when grown under an altered gravitational environment. Our observations clearly suggest that, on a cellular and structural level, the effects of clinorotation are not equivalent to microgravity. On a whole plant level, however, the effects are markedly similar. The predominant findings of the research show that both methods of altering gravity impact ethylene production and the extent to which starch is stored in the plastids of soybean cotyledons.

- Hilaire, E, AQ Paulsen, CS Brown, JA Guikema. 1995. Microgravity and clinorotation cause redistribution of free calcium in sweet clover columella cells. *Plant Cell Physiol.* 36(5), 831-837
- Hilaire, E, AQ Paulsen, CS Brown, JA Guikema. 1995. Effects of clinorotation and microgravity on sweet clover columella cells treated with cytochalasin D. *Physiologia Plantarum* 95, 267-273
- Hilaire, E, AQ Paulsen, CS Brown, JA Guikema. 1995. Cortical microtubules in sweet clover columella cells developed in microgravity. *Plant and Cell Physiology* 36(7), 1387-1392
- Hilaire, E, CS Brown, JA Guikema. 1995. The Fluid Processing Apparatus: From flight hardware to electron micrographs. *Journal of Gravitational Physiology*, 2(1), 165-166
- Hilaire, E, CS Brown, JA Guikema. 1995. Clinorotation affects soybean seedling morphology. *Journal of Gravitational Physiology*, 2(1) 149-150
- Hilaire, E, BV Peterson, JA Guikema, CS Brown. 1996. Clinorotation affects morphology and ethylene production in soybean seedlings. *Plant and Cell Physiology*, 37, 929-934
- Brown, CS, MM Sanwo, WC Piastuch, EM Stryjewski, CF Johnson, E Hilaire, JA Guikema. 199-. Altered carbohydrate metabolism and ethylene production in soybean seedlings grown in space. In preparation

B-PAC: Photosynthetic activity of *Brassica rapa*

Activities were focused on the use of *Brassica rapa* for monitoring the effects of microgravity on photosynthetic processes. Research activities proceeded along four lines. First, we established the methodology by which seeds could be placed, watered, and grown with the Plant Growth Facility. This was the first flight using this interesting hardware, and we took an approach whereby seeds were positioned in growth pouches, and the pouches inserted within florist foam blocks in the plant growth chambers. Methods were then developed by which water could be added to these blocks during spaceflight, such that all growth would occur after the seeds entered microgravity. Second, we established that this plant could be a useful model organism in the study of photosynthetic processes. We had earlier developed a number of immunological probes which recognized photosynthesis proteins, and these were examined using the *B. rapa* system. Interestingly, not all probes were useful within this context. Third, we examined a variety of environmental stresses (heat, high light, etc) that could be used as baseline measurements for understanding any impact of microgravity on photosynthesis. Finally, we recovered the cotyledon tissues after spaceflight, and we examined these for photosynthetic processes. Significantly, we observed that photosystem 1 was diminished in the spaceflight samples – this was apparent in both the rates of photosystem 1 and in the quantification of the photosystem 1 polypeptides.

- Adamchuck, N, J.A. Guikema. 1999, Structural and functional changes of photosynthetic membranes of *Brassica rapa* 6-9 days seedlings induced by microgravity. Journal of Gravitational Physiology. In press
- Adamchuck, N, S. Jiao, E. Hilaire, J.A. Guikema. 1999. Structure and function of *Brassica rapa* mesophyll cells in seedling grown under conditions of microgravity. Journal of Gravitational Physiology. In press
- Adamchuck, N, N.F. Mikhaylenko, E.K. Zolotareva, E. Hilaire, J.A. Guikema. 1999. Spaceflight effects on structural and biochemical parameters of *Brassica rapa* photosynthetic apparatus. Journal of Gravitational Physiology. In press
- Jiao, S, E. Hilaire, A.Q. Paulsen, E. Kordyum, J.A. Guikema. 1999. Ultrastructural observations of chloroplast morphology in space-grown *Brassica rapa* cotyledon tissue. Journal of Gravitational Physiology. In press
- Kordyum, E, G.I. Martin, Zaslavzky, V.A., S. Jiao, E. Hilaire, J.A. Guikema. 1999. DNA content and differentiation of root apical cells of *Brassica rapa* plants grown in microgravity. Journal of Gravitational Physiology. In press
- Jiao, S. 1999. Characterization of PSI structure and function in *Brassica rapa* grown under stress or in microgravity. Ph.D. Thesis. Kansas State University.

SOYPAT: The effects of microgravity on the interaction of a fungal root pathogen with soybean

Soybean was utilized in an experiment to examine the host defense responses of plants in microgravity. Two reasons motivated the choice of soybean. First, the available hardware mandated that this experiment be performed in darkness, and soybean had been extensively used to examine starch metabolism under these same conditions. Second, the pathogen *Phytophthora sojae* was known to infect soybean roots in a manner that is under gene-for-gene control. That is, certain races of the pathogen induce a susceptible response in specific soybean varieties, whereas other races induce a resistant response. This feature was exploited to allow us to examine both the infection of plant tissues by pathogens in space, and the induction of host defense responses based on the pathogenicity. We first designed the conditions of soybean growth and inoculation during spaceflight. We then developed the microscopic and analytical tools for the monitoring of infection. Our results clearly document an increased level of pathogenicity during spaceflight. Further experiments will be proposed to distinguish between (1) microgravity as it influences the plant deployment of general and induced defense responses and (2) microgravity as it increases the abilities of a pathogen to invade.

- Nedukha, OM, JE Leach, M Ryba-White, E Hilaire, J Guikema, EL Kordyum. 1998. Effects of microgravity on the susceptibility of soybean to *Phytophthora sojae*. Journal of Gravitational Physiology, 5, 143-144
- Leach, J. E., L.A. Lloyd, J. D. McGee, X. Wang, and J. A. Guikema. 1999. Trafficking of plant defense response compounds. In Delivery and Perception of Pathogen Signals in Plants. N. T. Keen, S. Mayama, J.E. Leach, S. Tsuyumu (eds), APS Press, Minneapolis. (in press).
- Nedukha, O, J.E. Leach, E. Kordyum, M. Ryba-White, J.A.. Guikema, E. Hilaire, A.Q. Paulsen, C.S. Brown, W. Piastuch. Effects of microgravity on ultrastructure of root meristem of soybean seedlings grown with a pathogenic fungus. Journal of Gravitational Physiology. In press